

Homologous Streptomycin Resistance Gene Present among Diverse Gram-Negative Bacteria in New York State Apple Orchards

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The streptomycin resistance gene of *Pseudomonas syringae* pv. *papulans* Psp36 was cloned into *Escherichia coli* and used to develop a 500-bp DNA probe that is specific for streptomycin resistance in *P. syringae* pv. *papulans*. The probe is a portion of a 1-kb region shared by three different DNA clones of the resistance gene. In Southern hybridizations, the probe hybridized only with DNA isolated from streptomycin-resistant strains of *P. syringae* pv. *papulans* and not with the DNA of streptomycin-sensitive strains. Transposon insertions within the region of DNA shared by the three clones resulted in loss of resistance to streptomycin. Colony hybridization of bacteria isolated from apple leaves and orchard soil indicated that 39% of 398 streptomycin-resistant bacteria contained DNA that hybridized to the probe. These included all strains of *P. syringae* pv. *papulans* and some other fluorescent pseudomonads and nonfluorescent gram-negative bacteria, but none of the gram-positive bacteria. The same-size restriction fragments hybridized to the probe in *P. syringae* pv. *papulans*. Restriction fragment length polymorphism of this region was occasionally observed in strains of other taxonomic groups of bacteria. In bacteria other than *P. syringae* pv. *papulans*, the streptomycin resistance probe hybridized to different-sized plasmids and no relationship between plasmid size and taxonomic group or between plasmid size and orchard type, soil association, or leaf association could be detected.

Resistance to streptomycin in *Pseudomonas syringae* pv. *papulans*, the causal agent of blister spot of apple, has been observed in New York, Ohio, and Michigan (4, 7a, 11a). Blister spot of apple is predominant on, but not restricted to, the cultivar Mutsu (3, 24). Previously, growers in New York successfully controlled the disease with streptomycin sprays. In 1985, incidents of control failure after the use of streptomycin were reported and streptomycin-resistant strains of *P. syringae* pv. *papulans* were isolated from several orchards (4). There was a significant correlation between the number of streptomycin sprays applied the previous season and the detection of streptomycin-resistant strains of *P. syringae* pv. *papulans* (4).

A 108-kb conjugative plasmid has been associated with streptomycin-resistant strains of *P. syringae* pv. *papulans* isolated in New York orchards (4). Although the plasmid content of *P. syringae* pv. *papulans* strains was found to be highly diverse, seven of seven streptomycin-resistant orchard strains and none of four streptomycin-sensitive orchard strains contained the 108-kb plasmid. Streptomycin resistance could be transferred from resistant to sensitive strains of *P. syringae* pv. *papulans* at high frequencies (2×10^{-2} per recipient cell), and transfer of resistance resulted in cotransfer of the 108-kb plasmid.

In this study, the streptomycin resistance gene of *P. syringae* pv. *papulans* was cloned and used to develop a DNA probe specific for streptomycin resistance in *P. syringae* pv. *papulans*. The probe was then used to study the occurrence of homologous DNA in other streptomycin-resistant bacteria isolated from apple leaves and soil of apple orchards.

MATERIALS AND METHODS

Development of DNA probe for the streptomycin resistance gene of *P. syringae* pv. *papulans*. Procedures for plasmid DNA isolation, agarose gel electrophoresis, restriction analysis, ligation, and transformation were described previously (1, 4, 12, 20). Total genomic DNA was isolated by the method of Lazo et al. (16). For Southern analysis, DNA was transferred to Gene Screen Plus-Hybridization Transfer Membrane (NEN Research Products) by alkaline transfer (23). DNA restriction fragments selected as probes were excised from low-melting-temperature agarose gels after electrophoresis and radiolabeled with ^{32}P by the randomized oligonucleotide labeling procedure of Feinberg and Vogelstein (8). DNA hybridizations were conducted by following recommended procedures of the manufacturer except that the hybridization buffer consisted of 1 M NaCl and 1% sodium dodecyl sulfate. Hybridizations and stringency washes were done at 65°C.

The mini-Tn10-*lacZ*-*kanR* gene fusion transposon Tn10-LK (10) was used to mutagenize pCPP503 by using a modification of the procedure of Way et al. (26) that excluded sodium pyrophosphate from the media used to select for transposon insertions. The *recA* nonsuppressor *Escherichia coli* KL16-99 (18) was used as the host strain of pCPP503 during transposon mutagenesis, the transposon vector was lambda gt7-*hiscI*857Pamb80nin (10), and transposase activity was provided by the plasmid pNK629 (10). After transposon mutagenesis, Tn10-LK insertions in pCPP503 were selected by combining all transfectants that grew on media containing 50 µg of kanamycin per ml (marker for Tn10-LK), and plasmid DNA was isolated, used to transform DH1 (9), and plated on Luria Bertani (LB) medium (20) amended with 100 µg of ampicillin and 50 µg of kanamycin per ml. Transformants were screened for their ability to grow on LB amended with 50 µg of streptomycin

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per ml, and plasmid DNA was isolated and mapped for restriction endonuclease sites.

Orchard sampling and isolation of bacteria. From each orchard, 500 to 1,000 leaves were collected at random, and six replicate 5-g subsamples of these leaves were placed in zip-lock bags with 50 ml of 0.05 M phosphate buffer, pH 6.5, and sonicated (Bransonic Ultrasonic Cleaner Model 8200; Branson Sonic Power Co., Shelton, Conn.) for 10 min. In each orchard, 40 soil samples consisting of 2.5-cm soil cores of the top 5 to 10 cm of soil were collected from under trees. Soil samples from a single orchard were combined and mixed, and three replicate 10-g subsamples were shaken in 100 ml of phosphate buffer for 30 min. Serial 100-fold dilutions of leaf and soil washes were plated on nutrient agar amended with 200 µg of cycloheximide per ml, nutrient agar amended with 200 µg of cycloheximide and 50 µg of streptomycin per ml, and on the *P. syringae* pv. *syringae*-selective KBC medium (21a) amended with 50 µg of streptomycin per ml.

After 48 h of incubation at 28°C, the total number of colonies was counted and one or two colonies representative of each morphological type present on isolation plates containing streptomycin were selected for further analysis. Isolates were maintained on 50-grid LB plates amended with 50 µg of streptomycin per ml.

Taxonomic identification of bacteria isolated from orchards. Tests for the production of cytochrome oxidase and gel liquefaction were performed as described by Jones (11), tests for production of levan from sucrose were as described by Lelliott et al. (17), and tests for ice nucleation at -3°C were as described by Legard and Hunter (16a).

Identification and analysis of strains with DNA homology to the streptomycin resistance gene of *P. syringae* pv. *papulans*. Colony hybridizations with DNA probe SMP3 (see Fig. 1) were performed with streptomycin-resistant bacteria isolated from orchards. Strains were transferred directly to Colony/Plaque Screen Hybridization Transfer Membrane (DuPont, NEN Research Products, Boston, Mass.) that was placed on the surface of an LB agar plate and incubated for 48 h at 28°C. Each filter contained colonies of *P. syringae* pv. *papulans* Psp36 (streptomycin resistant) as a positive control and Psp32 (streptomycin sensitive) as a negative control. Colonies were lysed by following the recommended procedures of the manufacturer. Hybridizations were performed as described above. Colony hybridizations were repeated once for all strains.

To study the occurrence of restriction fragment length polymorphism within the streptomycin resistance gene, plasmid DNA was isolated by the alkaline lysis miniprep procedure of Birnboim (1) from 103 strains that hybridized with the streptomycin resistance probe in colony hybridizations. Plasmid DNA was digested with *Ava*I, Southern transferred to hybridization membrane, and hybridized with probe SMP3 as described above.

Plasmid DNA of orchard strains was characterized by analysis of intact plasmid DNA. DNA was isolated by the Kado and Liu procedure (12), undigested DNA was electrophoresed in 0.7% agarose in Tris-acetate-EDTA buffer, stained with ethidium bromide, photographed, Southern transferred to hybridization membrane, and hybridized with probe SMP3 (see Fig. 1) as described above.

RESULTS

Development of DNA probe for the streptomycin resistance gene of *P. syringae* pv. *papulans*. The streptomycin resistance

gene was cloned from *P. syringae* pv. *papulans* Psp36 because it transfers streptomycin resistance to other *P. syringae* pv. *papulans* strains at a high frequency and contains fewer cryptic plasmids than most other strains of *P. syringae* pv. *papulans* (4). The 108-kb plasmid of strain Psp36 that is associated with streptomycin resistance has been designated pCPP501. The total plasmid DNA of Psp36 was partially digested with *Sau*3A and ligated into the alkaline phosphatase-treated *Bam*HI site of pBR322 (20). Ligated DNA was used to transform *E. coli* DH1 (9), and bacteria were plated on LB medium amended with 100 µg of ampicillin and 50 µg of streptomycin per ml. Characterization of plasmid DNA isolated from transformants resulted in the isolation of three DNA clones that conditioned streptomycin resistance in *E. coli*. The plasmids pCPP503, pCPP504, and pCPP505 contain inserts from strain Psp36 of approximately 8.6, 7.8, and 2.1 kb, respectively (Fig. 1).

DNA of these three clones was mapped by single, multiple, and sequential digestion with the restriction enzymes *Ava*I, *Bam*HI, *Eco*RI, *Hind*III, and *Pst*I (20). Mapping of the three clones indicated no obvious overlap between the clones. Comparison of *Sau*3A and double *Sau*3A-*Ava*I digestions indicated that all three clones shared an *Ava*I site within the same *Sau*3A fragment. Individual DNA restriction fragments were excised from gels, radiolabeled with ³²P, and used to confirm restriction maps by Southern analysis and to determine the regions of shared homology between the DNA clones. It was concluded that the three clones shared a 1-kb region of homology that included a single *Ava*I site (Fig. 1).

The specificity of individual DNA fragments for streptomycin resistance was tested by hybridizing the radiolabeled probes with a Southern blot of plasmid and total genomic DNA isolated from four streptomycin-sensitive orchard strains (Psp5, Psp21, Psp32, and Psp38) and seven streptomycin-resistant orchard strains (Psp33, Psp34, Psp35, Psp36, Psp37, Psp39, and Psp40) of *P. syringae* pv. *papulans* (4). SMP3, an approximately 500-bp *Bam*HI-*Ava*I fragment from the insert of pCPP505, did not hybridize with any DNA from four streptomycin-sensitive strains but did hybridize with DNA from all seven of the resistant strains. Fragment SMP3 was, therefore, used as a probe for the streptomycin resistance gene of *P. syringae* pv. *papulans* in orchard studies. In contrast, a 1.1-kb *Ava*I-*Bam*HI fragment from the insert of pCPP503, designated SMP1 (Fig. 1), was nonspecific for streptomycin resistance and hybridized with DNA from all 11 of the orchard strains.

Two transposon insertions in pCPP503 were mapped to the region of DNA homology shared by pCPP503, pCPP504, and pCPP505 (Fig. 1) and abolished the ability of the plasmid to condition resistance to streptomycin. Six transposon insertions in pCPP503 that had no effect on the ability of the plasmid to condition resistance to streptomycin were mapped to regions that did not share homology with the other plasmids (Fig. 1).

Isolation of bacteria from orchards. Bacteria were isolated from apple leaves and soil collected in 12 orchards in Wayne County, N.Y., 3 weeks after blossom petals had fallen from trees (7 June 1988). The relationship between the occurrence of the streptomycin resistance gene in bacterial populations and the presence of *P. syringae* pv. *papulans* was studied by collecting bacteria in 'Mutsu' orchards, where *P. syringae* pv. *papulans* is a common epiphyte, and in non-'Mutsu' orchards, where it is not likely to occur as an epiphyte. Twelve orchards were sampled, six 'Mutsu' orchards (three previously treated and three not treated with streptomycin)

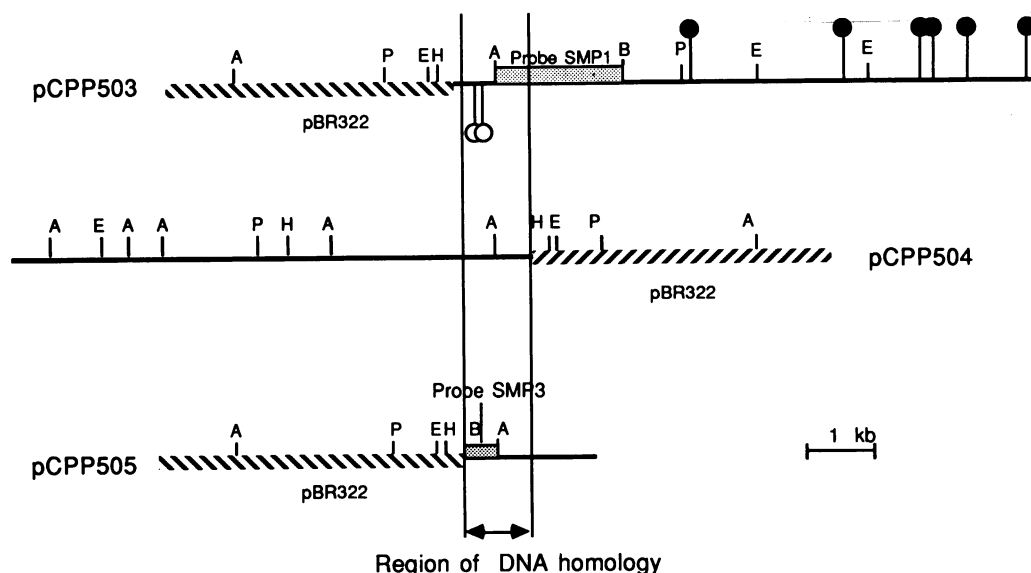


FIG. 1. Restriction endonuclease maps of three DNA clones obtained from *P. syringae* pv. *papulans* Psp36 that condition resistance to streptomycin in *E. coli*. The three maps are aligned with regard to a region of homology shared by all three plasmids. Restriction endonuclease recognition sites: A, *Ava*I; B, *Bam*HI; E, *Eco*RI; H, *Hind*III; P, *Pst*I. Hatched lines represents pBR322. Open circles below line represent mapped locations of transposon Tn10-LK (10) insertions resulting in sensitivity to streptomycin, and closed circles above line represent mapped locations of transposon insertions not affecting resistance to streptomycin.

and six non-'Mutsu' orchards (three previously treated and three not treated with streptomycin).

The percentage of bacteria isolated from apple leaves that were resistant to streptomycin ranged from 33.9 to less than 0.001%, with a median value of 1.1% and a mean of 0.7%. In orchard soils, the percentage of streptomycin-resistant bacteria ranged from 23.6 to 0.1%, with a median value of 1.0% and a mean of 1.5%. No significant relationships between orchard type or previous streptomycin usage and the percentage of bacteria that were resistant to streptomycin could be detected.

On the basis of a gram-differentiation test (25) and the ability of bacteria to fluoresce on B medium of King et al. (13), streptomycin-resistant bacteria were placed in three general taxonomic groups: gram-positive bacteria, nonfluo-

rescent gram-negative bacteria, and fluorescent pseudomonads. *P. syringae* pv. *papulans* was distinguished from other fluorescent pseudomonads by negative reactions for production of cytochrome oxidase, gel liquefaction, levan production from sucrose, and ice nucleation at -3°C (2, 7). The pathogenicity of six representative strains characterized as *P. syringae* pv. *papulans* by these tests was determined by inoculation onto immature apple 'Mutsu' fruit in the orchard, and all caused typical blister-spot symptoms.

Streptomycin-resistant bacteria isolated in orchards hybridized with the DNA probe for the streptomycin resistance gene of *P. syringae* pv. *papulans*. Thirty-nine percent of 398 streptomycin-resistant bacteria isolated from the orchards hybridized with the streptomycin resistance probe SMP3 of *P. syringae* pv. *papulans* in colony hybridizations (Table 1).

TABLE 1. Effect of streptomycin usage and cultivar on the number of bacteria isolated from apple leaves and orchard soil that hybridized with the streptomycin resistance probe SMP3 of *P. syringae* pv. *papulans*

Bacteria isolated from:	Orchard type	No. of strains that hybridized with SMP3/no. of strains tested ^a				
		<i>P. syringae</i> pv. <i>papulans</i>	Fluorescent pseudomonads	Nonfluorescent gram-negative bacteria	All gram-negative bacteria	Gram-positive bacteria
Leaf	'Mutsu,' treated ^b	28/28 (100)	16/16 (100)	35/36 (97)	79/80 (99)	0/5 (0)
	'Mutsu,' not treated	8/8 (100)	3/7 (43)	5/26 (19)	16/41 (39)	0/23 (0)
	Non-'Mutsu,' ^c treated	0/0	2/7 (29)	1/20 (5)	3/27 (11)	0/17 (0)
	Non-'Mutsu,' not treated	0/0	2/6 (33)	14/29 (48)	16/35 (46)	0/10 (0)
	Total	36/36 (100)	23/36 (64)	55/111 (49)	111/183 (62)	0/55 (0)
Soil	'Mutsu,' treated	0/0	0/9 (0)	23/27 (85)	23/26 (64)	0/5 (0)
	'Mutsu,' not treated	0/0	1/8 (13)	4/17 (23)	5/25 (20)	0/8 (0)
	Non-'Mutsu,' treated	0/0	9/21 (41)	17/28 (61)	26/49 (53)	0/1 (0)
	Non-'Mutsu,' not treated	0/0	8/19 (42)	6/11 (54)	14/30 (47)	0/6 (0)
	Total	0/0	18/57 (32)	50/83 (60)	68/140 (49)	0/20 (0)

^a Numbers in parentheses indicate the percentage of positive strains.

^b Mutsu cultivar treated with streptomycin in 1987.

^c Non-'Mutsu' is either 'Ben Davis,' 'Empire,' 'Ida Red,' 'Paula Red,' or 'Red Delicious.'

TABLE 2. Amount of restriction fragment length polymorphism^a in the SMP3 streptomycin resistance region of bacteria isolated from apple leaves and orchard soils

Taxonomic group	No. of strains with SMP3 polymorphism/no. of strains tested	
	Leaf	Soil
<i>P. syringae</i> pv. <i>papulans</i>	0/8 (0) ^b	
Fluorescent pseudomonads	0/17 (0)	1/12 (8)
Nonfluorescent gram-negative bacteria	9/43 (21)	11/23 (48)
All	9/68 (13)	12/35 (32)

^a Plasmid DNA was digested with *Ava*I, separated by agarose gel electrophoresis, Southern blotted, and hybridized with labeled probe SMP3 from *P. syringae* pv. *papulans*.

^b Numbers in parentheses indicate percentage of positive strains.

Both leaf epiphytes and soil bacteria hybridized with the probe. All streptomycin-resistant strains of *P. syringae* pv. *papulans*, some of the fluorescent pseudomonads and nonfluorescent gram-negative bacteria, and none of the streptomycin-resistant gram-positive bacteria isolated from the orchards hybridized with the probe (Table 1). Hybridizing fluorescent pseudomonads included both oxidase-positive and -negative strains.

P. syringae pv. *papulans* was never recovered from soil or from non-'Mutsu' orchards (Table 1). Strains of *P. syringae* pv. *papulans* resistant to streptomycin were isolated from 'Mutsu' orchards that had no previous history of streptomycin use (Table 1). Although *P. syringae* pv. *papulans* was not detected in non-'Mutsu' orchards, several streptomycin-resistant strains of fluorescent pseudomonads and nonfluorescent gram-negative bacteria from these orchards hybridized with the streptomycin resistance probe of *P. syringae* pv. *papulans* (Table 1). In 'Mutsu' orchards sprayed with streptomycin, a much higher percentage of the fluorescent pseudomonads and nonfluorescent gram-negative bacteria isolated from leaves hybridized with the streptomycin resistance probe than in orchards not sprayed with streptomycin (Table 1). This relationship was not observed among soil bacteria (Table 1).

Characterization of plasmid DNA from bacteria that hy-

bridized with the streptomycin resistance probe. The amount of restriction fragment length polymorphism observed in DNA hybridizing to SMP3 increased in bacteria with increasing phylogenetic distance from *P. syringae* pv. *papulans* (Table 2). Among the 103 strains that hybridized to the streptomycin resistance probe that were analyzed, there was a greater amount of polymorphism among nonfluorescent gram-negative bacteria than among fluorescent pseudomonads (Table 2). There was also a greater amount of polymorphism in bacteria isolated from soil than from leaves (Table 2). *P. syringae* pv. *papulans* DNA digested with *Ava*I and hybridized with probe SMP3 resulted in the hybridization of a single band of 1.6 kb. Hybridization of DNA isolated from nonfluorescent gram-negative orchard bacteria resulted in the hybridization of a single band of either 1.6, 2.9, or 1.3 kb.

Plasmid DNA from 120 strains that hybridized with probe SMP3 (Fig. 1) was characterized by hybridization analysis of intact plasmid DNA. In general, orchard bacteria recovered in this study contained one to several plasmids of different sizes (Fig. 2). The streptomycin resistance probe (SMP3) hybridized with two plasmids in *P. syringae* pv. *papulans* Psp36, i.e., pCPP501 and a smaller plasmid (Fig. 2, lane 2). In contrast, the probe hybridized to a single plasmid in eight other *P. syringae* pv. *papulans* strains. In seven of eight *P. syringae* pv. *papulans* strains, the plasmid that hybridized to the probe appeared to be of the same size as pCPP501; however, in one strain the hybridizing plasmid appeared to be slightly smaller than pCPP501 (Fig. 2, lane 4). In bacteria other than *P. syringae* pv. *papulans*, the streptomycin resistance probe usually hybridized to one plasmid per strain (Fig. 2, lanes 4 to 14), the hybridizing plasmids were of several different sizes (Fig. 2, lanes 4 to 14), and no relationship between plasmid size and taxonomic group or between plasmid size and orchard type, soil association, or leaf association could be detected.

Streptomycin-resistant strains of *P. syringae* pv. *papulans* have also been isolated in Ohio. Although some of these strains contain DNA that hybridizes to probe SMP3 (data not shown), others do not hybridize to the probe (Fig. 2, lane 16). Probe SMP3 hybridized to streptomycin-resistant *P. syringae* pv. *syringae* strains isolated from brown-spot lesions in New York (Fig. 2, lane 15).

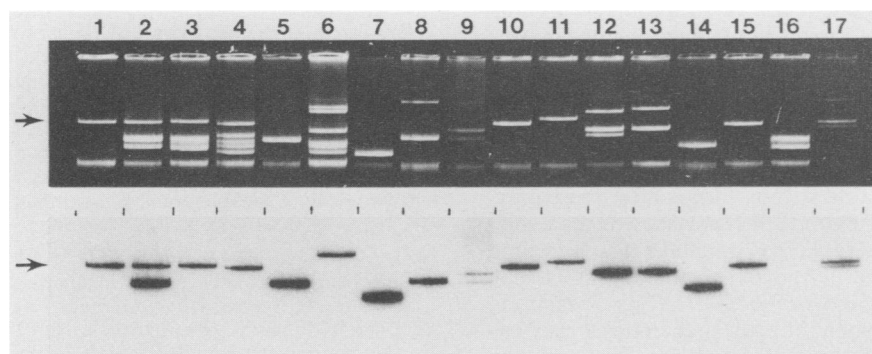


FIG. 2. Agarose gel electrophoresis and corresponding autoradiograph of plasmid DNA from streptomycin-resistant bacteria isolated from apple orchards. Arrow indicates pCPP501 which shows homology to DNA probe in lane 1 of corresponding autoradiograph. Bottom band in agarose gel common to all lanes is linear DNA artifact. Lanes: 1, pCPP501; 2, *P. syringae* pv. *papulans* Psp36, 3 to 14; plasmid DNA of strains of bacteria isolated in apple orchards in this study, namely, *P. syringae* pv. *papulans* (lanes 3 and 4), oxidase-negative fluorescent pseudomonads isolated from leaves (lanes 5 to 7), oxidase-positive fluorescent pseudomonad isolated from leaves (lane 8), gram-negative nonfluorescent bacteria isolated from leaves (lanes 9 to 12), and gram-negative nonfluorescent bacteria isolated from soil (lanes 13 and 14); 15, *P. syringae* pv. *syringae* B86-6 isolated from bean plant brown-spot lesion in New York; 16, streptomycin-resistant *P. syringae* pv. *papulans* Psp46 isolated from 'Mutsu' apple in Ohio; and 17: oxidase-positive *P. syringae* isolated from leaves.

DISCUSSION

These results show that DNA associated with streptomycin resistance in the plant-pathogenic bacteria *P. syringae* pv. *papulans* is part of the gene pool of a diverse group of gram-negative bacteria in the orchard environment. There have been numerous demonstrations of the in vitro and in planta transfer of resistance plasmids between plant pathogenic bacteria and bacteria associated with plants and animals under laboratory or controlled experimental conditions (5, 14, 15, 19, 22). The presence of homologous DNA associated with streptomycin resistance among diverse gram-negative bacteria in an orchard environment indicates that the transfer of drug resistance genes between plant-associated bacteria occurs in agricultural ecosystems. The streptomycin resistance gene(s) present in gram-positive bacteria in orchard environments appears to be unrelated to that of *P. syringae* pv. *papulans*.

The specificity of probe SMP3 to streptomycin-resistant strains of *P. syringae* pv. *papulans* and evidence from mapping data (Fig. 1) indicate that SMP3 is most likely part of the resistance gene of *P. syringae* pv. *papulans* or DNA tightly linked to it. Since SMP3 hybridizes with two plasmids in Psp36 (Fig. 2), it is possible that clones pCPP503, pCPP504, and pCPP505 contain DNA from different regions of the genome. However, SMP3 hybridizes with a single *Ava*I fragment from Psp36 (data not shown). pCPP503, pCPP504, and pCPP505 share a relatively small region of DNA homology, and SMP3 hybridizes to this shared region of DNA homology in all three plasmids. In addition, transposon insertions within that region result in a loss of resistance to streptomycin.

It is surprising that probe SMP1 shows a complete lack of specificity for streptomycin resistance, given its close physical proximity to SMP3 (Fig. 1). SMP1 hybridized with three to four DNA fragments per strain and may represent part of an insertion sequence.

Field observations and the data presented in this paper indicate that the plasmid-mediated spread of antibiotic resistance resulted in the development of streptomycin-resistant *P. syringae* pv. *papulans* in New York. The use of streptomycin in orchards to control bacterial plant diseases other than blister spot of apple, such as fire blight caused by *Erwinia amylovora*, may have resulted in the selection of populations of nonpathogenic bacteria that were resistant to streptomycin. The subsequent use of streptomycin to control blister spot of apple may have then resulted in the transfer of a streptomycin resistance gene to *P. syringae* pv. *papulans* from resistant bacteria that was rapidly spread through the population of *P. syringae* pv. *papulans* on a plasmid. Several observations support this hypothesis. Resistance to streptomycin in *P. syringae* pv. *papulans* occurred rapidly in New York, within 4 years after streptomycin started being used to control the disease. Streptomycin-resistant strains of *P. syringae* pv. *papulans* also appeared rapidly in Ohio. However, the lack of homology between strain Psp46 from Ohio and probe SMP3 (Fig. 2, lane 16) indicate that the development of resistance in this strain was the result of an independent event. Secondly, streptomycin-resistant bacteria with homology to the streptomycin resistance probe of *P. syringae* pv. *papulans* were routinely isolated from orchard sites where *P. syringae* pv. *papulans* could not be detected and would not normally occur, including non-'Mutsu' orchards and orchard soils (Table 1). Thirdly, in the *P. syringae* pv. *papulans* strains isolated in New York, streptomycin resistance is associated with a

single plasmid (Fig. 2) (4). The lack of plasmid heterogeneity associated with resistance suggests that the spread of this plasmid is a relatively recent event. Although streptomycin resistance is associated with a single plasmid, the cryptic plasmid content of streptomycin-resistant *P. syringae* pv. *papulans* strains is highly diverse (4), indicating that resistance has probably not resulted from the spread of a single strain. In contrast, bacteria other than *P. syringae* pv. *papulans* had a much greater diversity of plasmids associated with streptomycin resistance (Fig. 2). In Michigan, unlike New York, streptomycin resistance in *P. syringae* pv. *papulans* is associated with plasmids of several different sizes (11b).

The orchards sampled in this study were in close proximity to other orchards, providing significant opportunity for the transfer of bacteria by humans, birds, insects, or wind from orchards receiving different streptomycin treatments and planted with different cultivars. This may explain the occurrence of streptomycin-resistant *P. syringae* pv. *papulans* and other streptomycin-resistant leaf epiphytic bacteria in orchards not treated with streptomycin. Despite the close proximity of non-'Mutsu' orchards to 'Mutsu' plantings, *P. syringae* pv. *papulans* could not be detected in non-'Mutsu' orchards.

The mechanism by which resistance to streptomycin is conditioned in *P. syringae* pv. *papulans* Psp36 is not known. However, the relatively low level of resistance of this strain (4) and the plasmid-borne nature of the resistance suggest that resistance is due to modification or destruction of streptomycin by periplasmic enzymes rather than alteration of the binding affinity of ribosomal proteins for streptomycin (6). The homology of probe SMP3 to other cloned streptomycin resistance genes or transposable elements has not yet been determined.

DNA homologous to SMP3 appears to be common and widespread among streptomycin-resistant plant pathogenic bacteria. In addition to the *P. syringae* pv. *syringae* strain reported here, SMP3 has shown homology with *P. syringae* strains isolated in Michigan, Ohio, and Georgia (21b). *P. syringae* pv. *papulans* Psp36 has also been reported to contain DNA homologous with the streptomycin resistance gene of *Xanthomonas campestris* pv. *vesicatoria* BV5-4a (21).

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